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Translation of Final Report

An Acute Inhalation Toxicity Study of [redacted] in Rats

(Study No.: [redacted])

LSI Medience Corporation

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Translation Statement

The following study was conducted in the Kashima Laboratory of LSI Medience Corporation. The original final report was written in Japanese. I hereby declare that this translation report faithfully reflects the original final report as accurately as possible.

Information of the original study
Title: An Acute Inhalation Toxicity Study of [redacted] in Rats
Study No.: [redacted]

Study No. for translation:
[redacted]

Translator:

[redacted] Date: June 19, 2017

Kiyoshi Wako
Kashima Safety Assessment Department,
Nonclinical Research Center, Drug Development Service Segment,
LSI Medience Corporation

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[redacted]

Final Report

An Acute Inhalation Toxicity Study of [redacted] in Rats

(Study No.: [redacted])

LSI Medience Corporation

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[redacted]

1. Statement

Title: An Acute Inhalation Toxicity Study of [redacted] in Rats

Study No.: [redacted]

This study was carried out in compliance with the following regulation.

OECD Principles of Good Laboratory Practice (as revised in 1997)

Study director:

Signed _____ Date: June 5, 2017

Kiyoshi Wako
Kashima Safety Assessment Department,
Nonclinical Research Center, Drug Development Service Segment,
LSI Medience Corporation

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2. Contents

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3. Study Outline

3.1 Title

An Acute Inhalation Toxicity Study of [redacted] in Rats

3.2 Study No.

[redacted]

3.3 Purpose

The purpose of this study is to assess the acute inhalation toxicity of [redacted] by exposing it once to rats.

3.4 Guideline

OECD Guideline for the Testing of Chemicals (No. 403, September 7, 2009) applied mutatis mutandis

3.5 GLP

OECD Principles of Good Laboratory Practice (as revised in 1997)

3.6 Sponsor

[redacted]

3.7 Contract research organization

LSI Medience Corporation
1-13-4, Uchikanda, Chiyoda-ku, Tokyo 101-8517, Japan

3.8 Test facility

Kashima Laboratory,
LSI Medience Corporation
14-1, Sunayama, Kamisu-shi, Ibaraki 314-0255, Japan

3.9 Study director

Kiyoshi Wako
Kashima Safety Assessment Department,
Nonclinical Research Center, Drug Development Service Segment,
LSI Medience Corporation
14-1, Sunayama, Kamisu-shi, Ibaraki 314-0255, Japan

[redacted]

3.10 Main study contributors

(Animal receipt, quarantine and acclimation)
Masanori Hoshino, Takeshi Kawasuso
(Animal allocation, identification of animals)
Kiyoshi Wako, Tomohiro Ikeda
(Inhalation exposure and exposure concentration measurement)
Masanori Hoshino
(Clinical observation, body weight measurement)
Masanori Hoshino, Tomohiro Ikeda
(Necropsy)
Masanori Hoshino, Tomohiro Ikeda

3.11 Study schedule

Study initiation:	March 31, 2017
Animal receipt:	April 5, 2017
Animal allocation:	April 10, 2017
Inhalation exposure (initiation of experiment):	April 11, 2017
End of observation (completion of experiment):	April 25, 2017
Completion of the study:	Date signed by the study director

3.12 Archiving

Study-related documents described in the next section will be retained in a document archive room of the test facility. The archiving duration is 5 years after preparation of the final report, and subsequent retention will be determined after consultation with the sponsor.

3.13 Archived materials

(1) Study protocol
(2) Study protocol amendment
(3) Documents on test substance
(4) Documents on used animals
(5) Documents on test results
(6) Record documents such as communication documents
(7) Final report

3.14 Others

Conduct of this study was reviewed by Animal Experimentation Committee and approved

[redacted]

by the director of Nonclinical Research Center (Approval number: 2017-0198), according to "Guidelines for Animal Studies (Guidelines of Nonclinical Research Center)."

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[redacted]

4. Study Director Signature

Title: An Acute Inhalation Toxicity Study of [redacted] in Rats

Study No.: [redacted]

Study director:

Signed _____ Date: June 5, 2017

Kiyoshi Wako
Kashima Safety Assessment Department,
Nonclinical Research Center, Drug Development Service Segment,
LSI Medience Corporation

5. Summary

Acute inhalation toxicity of [redacted] was evaluated in rats.

The test substance was exposed once for 4 hours to Crl:CD(SD) rats at the target concentration of 2500 ppm by nose-only inhalation exposure. Observation period was set at 14 days; the rats were observed their clinical signs, weighed body weight, and subjected to necropsy after the observation period.

The actual exposure concentration was 2520 ppm. There were no changes to be affected to the study results in temperature or relative humidity of the test atmosphere.

In the results of exposure, no dead animals were noted. There were no abnormalities in clinical signs. Increase of the body weights was observed after day 4 (day of exposure designated as day 1). No gross abnormalities were observed at the necropsy after the observation period.

From the above results, it is concluded that the 50% lethal concentration (LC50) of [redacted] is more than 2520 ppm as the actual exposure concentration.

[redacted]

6. Materials and method

6.1 Test substance

6.1.1 Name

[redacted]

6.1.2 Chemical name

2,3,3,3-tetrafluoro-2-(trifluoromethyl) propanenitrile

6.1.3 CAS No.

42532-60-5

6.1.4 Molecular weight

195.04

6.1.5 Appearance

Colorless gas

6.1.6 Lot No.

13

6.1.7 Supplier

[redacted]

6.1.8 Storage condition

Room temperature (actual range: 18.0°C–22.7°C, acceptable range: 10°C–30°C)

6.1.9 Handling precautions

The following items were worn: safety glasses, a mask, and rubber gloves.

6.1.10 Stability confirmation

The sponsor indicates that the expiry date of the test substance in this study is to be 5 years from the shipping date (February 20, 2017), and the test substance was used within the expiry date.

6.1.11 Handling of remaining test substance

All remaining test substance was discarded at the test facility.

6.2 Study animals

6.2.1 Species

Rats

[redacted]

6.2.2 Strain
Crl:CD(SD)

6.2.3 Rationale for strain selection
This strain is widely used in toxicity study using rodents, and there are abundant historical data.

6.2.4 Microbial level
SPF

6.2.5 Supplier
Charles River Laboratories Japan, Inc.

6.2.6 Age at receipt
7 weeks of age

6.2.7 Gender and number of animals purchased
8 males and 8 females

6.2.8 Quarantine/acclimatization
Quarantine period was set for 5 days from animal receipt. The following examinations were conducted during the quarantine period according to Section 6.6:
· Clinical observation (once a day)
· Measurement of body weight (at animal receipt and at the end of quarantine)
The animals were acclimatized from animal receipt to the day before the exposure.
It was confirmed that there were no abnormalities in clinical sign and body weights increased during the quarantine/acclimatization period.

6.2.9 Week of age at exposure
8 weeks of age

6.2.10 Body weight range at exposure
Males: 305.2–323.6 g, females: 211.8–234.6 g
It was confirmed that the body weight ranges were within ±20% of mean body weight of males and females on the day of exposure.

6.2.11 Allocation to group
(1) Implementation day
The day designated in Section 3.11

[redacted]

(2) Animal selection

All males and females were selected for the animal assignment based on the examinations results during the quarantine and acclimatization periods (Section 6.2.8).

(3) Animal assignment

Animals were allocated to study group by the body weight stratified random sampling method based on the body weight on the allocation day. This study used 6 males and 6 females.

6.2.12 Acclimatization to restraint tube

On the day of allocation to group, the animals after completion of allocation were held in a restraint tube for nose-only inhalation exposure (Muenster Ltd.) for 30 minutes to acclimatize to the restraint tube.

6.2.13 Identification of animals

Individual identification was performed as the following:

Before allocation: The last 1 digits of each animal number before allocation were written down on the tail with a permanent marker.

After allocation: Back subcutaneous implant of a microchip (IPT-300: BioMedic Data Systems, Inc.) in which an animal number was registered; a microchip reader (DAS-5002: BioMedic Data Systems, Inc.)

Animal numbers before allocation were 19001–19008 in males and 59001–59008 in females.

6.2.14 Handling of surplus animals

The animals that had not been used for the study were euthanized 6 days after the exposure, by exsanguination via the abdominal aorta under anesthesia with an intraperitoneal injection of thiopental sodium (Ravonal: :Mitsubishi Tanabe Pharma Corp.) .

6.3 Animal housing

6.3.1 Housing room

Rat/Mouse /Guinea pigs/Rabbits housing room (Room 7117)

6.3.2 Housing environment

6.3.2.1 Temperature

Actual range: 21.1°C–22.4°C, acceptable range: 19.0°C–25.0°C

6.3.2.2 Relative humidity

Actual range: 48.7%–63.3%, acceptable range: 35.0%–75.0%

[redacted]

6.3.2.3 Air exchange

6 to 20 times per hour: all fresh air supply

The ventilation frequency is periodically measured twice a year. It was confirmed to be within the acceptable range as described in the standard operating procedure (SOP) of the test facility.

6.3.2.4 Lighting time

12-hour light-on per day (7:00–19:00)

6.3.3 Housing materials

6.3.3.1 Cages

Polycarbonate cages (W 220 × D 380 × H 183 mm: Tokiwa Kagaku Kikai Co., Ltd.)

Sterilization before use: Autoclave sterilization

Frequency of replacement: 4–5 days interval

6.3.3.2 Feeders

Stainless steel feeders for pellet food (Tokiwa Kagaku Kikai Co., Ltd.)

Sterilization before use: Autoclave sterilization

Frequency of replacement: At the time of a cage replacement

6.3.3.3 Watering bottles

Polycarbonate water bottles (700 mL; Tokiwa Kagaku Kikai Co., Ltd.)

Sterilization before use: Autoclave sterilization

Frequency of replacement: At the time of a cage replacement

6.3.3.4 Racks

Stainless steel racks (Tokiwa Kagaku Kikai Co., Ltd.)

Disinfection before usage: Wiping with sodium hypochlorite solution

Disinfection frequency: 4–5 days interval

6.3.3.5 Enrichment

In order to improve animal welfare, the following enrichment products were used.

Items: Paper chips (Diamond Twists: Envigo, Paper Clean: Japan SLC, Inc.)

Sterilization before use: Autoclave sterilization

Frequency of replacement: At the time of a cage replacement

6.3.4 Number of animals in a cage

2 animals/cage (same sex)

[redacted]

6.3.5 Bedding

6.3.5.1 Type

Bedding for laboratory animals (Beta Chip: Charles River Laboratories Japan, Inc.)
Sterilization before use: Autoclave sterilization
Frequency of replacement: At the time of a cage replacement

6.3.5.2 Confirmation check

Analysis results of contaminants such as residual pesticides were obtained from the supplier of bedding, and the contaminant level was confirmed to meet the criteria specified in the SOP of the test facility.

6.3.6 Food

6.3.6.1 Type

Pellet food for laboratory animals (MF: Oriental Yeast Co., Ltd.)

6.3.6.2 Feeding methods

Provided *ad libitum*, except that no food was provided during inhalation exposure.
Food was changed the same time as the cage replacement.

6.3.6.3 Confirmation check

Analysis results were obtained from the food supplier, and the levels of contaminants such as residual pesticides in used lot (170118) was confirmed to meet the criteria specified in the SOP of the test facility.

6.3.7 Drinking Water

6.3.7.1 Type

Tap water filtered through a 5-μm pore size filter followed by irradiation with ultraviolet

6.3.7.2 Water supply methods

Provided *ad libitum*, except that no water was provided during inhalation exposure.
Water was changed the same time as the water bottle replacement.

6.3.7.3 Analyses

Water quality was periodically (twice a year) analyzed by an external agency (Ibaraki Pharmaceutical Association Inspection Center). The values obtained from the analysis were confirmed to meet the criteria specified in the SOP of the test facility.

6.4 Exposure

6.4.1 Exposure route and method

Inhalation exposure (nose-only inhalation exposure) was selected as exposure route to

assess the safety of the test substance when it will be exposed to human by inhalation. Fig. 1 shows the inhalation exposure system used in this study. The animals individually held in the restraint tube (Muenster Ltd.) were subjected to the nose-only inhalation exposure using a flow-past nose-only inhalation exposure chamber (hereinafter abbreviated as "chamber", Muenster Ltd.) according to the method widely used in the similar tests. The chamber was constructed from stackable tiers, which has 16 exposure ports per tier. This study used the chamber constructed from single tier. The flow rate of test atmosphere supply to the chamber was set at 1 L/min/exposure-port (acceptable range: 1.0–1.4 L/min/exposure-port). The flow rate of the chamber exhaust was set at approximately 10% lower than the test atmosphere supply, because the inner pressure of the chamber has to be positive to ensure a reliable exposure.

6.4.2 Exposure duration and frequency

Inhalation exposure was conducted once for 4 hours in accordance with the description of the guideline applied mutatis mutandis.

6.4.3 Test atmosphere generation and exposure

6.4.3.1 Preparation of test substance

The test substance supplied from the supplier was used as is.

6.4.3.2 Test atmosphere generation

The test atmosphere at the target concentrations was prepared by mixing air and constant flow rate of the test substance measured by a mass-flow controller after adjusting to appropriate pressure with a pressure regulator. The test atmosphere was continuously supplied to the chamber to expose it. The air from the chamber was exhausted to outside air with processing with a filter.

6.4.3.3 Inhalation exposure

The rats held in the restraint tubes were connected to the chamber to start exposure 5 minutes or more (actual time: 15 minutes), which is sufficient duration for concentration equilibrium, elapsed after supplying the test atmosphere to the chamber. The rats were dismounted from the chamber to terminate exposure 4 hours after the start of exposure.

6.4.4 Exposure related measurements

6.4.4.1 Exposure concentration temporal monitor

Hydrocarbon concentration in the test atmosphere was continuously monitored with a hydrocarbon monitor (HCM-1B: Shimadzu Corp.) during exposure. Temporal variability of the concentration was confirmed by recording the output signal of the hydrocarbon monitor.

[redacted]

6.4.4.2 Nominal concentration

The amount of test substance used during the test atmosphere generation was obtained by weight measurement. The total air volume supplied to the chamber was calculated by multiplying the elapsed time of the test atmosphere generation to the air flow rate of the test atmosphere supply (minimum unit: 0.1 L/min). The nominal concentration (minimum unit: 10 ppm) was calculated with the following equation from the amount of the test substance used and the total air volume supplied to the chamber.

$$C_n = \frac{\frac{W_{ts}}{MW} \times V_{mol}}{V_{air}} \times 10^6$$

C_n: Nominal concentration (minimum unit: 10 ppm)
W_{ts}: Used test substance weight (minimum unit: 1 g)
MW: Molecular weight (195.04)
V_{mol}: 1 molar gas volume (24.8L, 25°C)
V_{air}: Air volume (minimum unit: 0.1 L)

6.4.4.3 Measurement of exposure concentration

The test substance in the test atmosphere was quantified by a gas chromatography (hereinafter abbreviated as "GC"), then, the exposure concentration was calculated.

6.4.4.3.1 Analytical method

(1) Equipments

GC:	GC-14B (Shimadzu Corp.)
Recorder:	D-7500 (Hitachi, Ltd.)
Syringe:	1 mL gastight syringe (#1001: Hamilton Company)
Sampling bag:	1.56 L of inner volume (actual inner volume, Smart bag: GL Sciences Inc.)

(2) GC condition

Detector:	Hydrogen flame ionization detector
Columm:	5% SE-30, Uniport HP (100 - 120 mesh), inside diameter 3.2 mm, length 2 m, glass column
Injection temperature:	150°C
Detector temperature:	150°C
Column temperature:	60°C
Carrier gas:	Nitrogen
Carrier gas flow:	40 mL/min
Injection volume:	0.5 mL

(3) Preparation of standard gas

· Injecting the test substance with a gas tight syringe into the sampling bag according to

the following table

· Filling up the sampling bag with the pressurized air at 0.01 MPa or less

The concentration of the gas was calculated from injected volume of the test substance, inner volume of the sampling bag (minimum unit: 10 ppm).

Standard gas abbreviation	Injection volume of the test substance (mL)	Test substance concentration (ppm)
ST-1	1.6	1030
ST-2	3.9	2500
ST-3	5.5	3530

(4) Confirmation of specificity

· Analyzing air in the experimental room under the GC condition described in the above section

· Checking whether there is interference peak at the retention time of the derived peak from the test substance on the obtained chromatogram or not

Acceptable range: the interference peak to be less than 5% of peak area derived from the test substance of ST-1

Results: There was no interference peak, and it was confirmed that the result was within the acceptable range.

(5) Preparation of calibration curve

Each standard gas was injected once to GC and analyzed. The calibration curve was constructed by a linear regression formula obtained from the concentrations and peak areas of the standard gases by the least square method. The acceptable range to the linearity of the calibration curve was set that the coefficient of correlation of the calibration curve to be more than 0.995, and it was confirmed that the result met the acceptable range (0.999).

6.4.4.3.2 Sampling and analysis of the test atmosphere

(1) Equipments

Syringe: 1 mL gas tight syringe (#1001: Hamilton Company)

Sampling bag: 1.56 L of inner volume (actual inner volume, Smart bag: GL Sciences Inc.)

Vacuum sampling container:

A container for sampling the test atmosphere to the sampling bag its inside by reducing inner pressure (in-house made)

Vacuum pump: General-purpose pump

[redacted]

(2) Sampling

Sampling position: Exposure port of the chamber

Time of sampling:

30 minutes, 2 hours, and 3 hours 30 minutes after the start of exposure (actual time: on time, acceptable: before and after 5 minutes)

Number of sample: 3 samples simultaneously sampled from 3 exposure-ports at 30 minutes after the start of exposure

1 sample/time point on another sampling points

Sampling method: The sampling bag was put into the vacuum sampling container, and connected to the exposure port. The test atmosphere was sampled to the sampling bag by reducing inner pressure of the container with the vacuum pump.

(3) Analysis and exposure concentration calculation

Analysis subject: Analytical sample

Number of analysis: 1 analysis/sample

Exposure concentration calculation:

Calculating of exposure concentration from the calibration curve (minimum unit: 10 ppm)

(4) Evaluation of exposure concentration

(a) Calculated items

Exposure concentration:

Mean exposure concentration

(Mean concentration of 3 samples/time point is designated as representative concentration on its time point.)

Concentration homogeneity:

Coefficient variation of exposure concentrations from 3 points measurements at 30 minutes after the start of exposure (minimum unit: 0.1%)

Concentration stability:

Coefficient variation of exposure concentration of 3 times sampling (minimum unit: 0.1%)

Relative error of each measuring value to mean concentration of 3 times sampling (minimum unit: 0.1%)

(b) Criteria values of temporal concentration stability

Concentration homogeneity: Coefficient variation: 15% or below

Concentration stability: Coefficient variation: 10% or below

Relative error: ±10% or below

$$R.E.(\%) = \left(\frac{C_m - C_{Ave}}{C_{Ave}}\right) \times 100$$

$R.E.$: Relative error
C_m: Measured concentration (ppm)
C_{Ave}: Mean concentration (ppm)

6.4.4.4 Air flow rate of chamber

Time of measurement: Start of test atmosphere generation, start of exposure, 1, 2, 3, and 4 hours after start of exposure, termination of test atmosphere generation (actual: on time, acceptable: before and after 5 minutes)
Measurement object: Air supply and exhaust of chamber
Minimum unit: 0.1 L/min
Calculation items: Air change frequency of the chamber
Method: According to the following formula

$$F_{Air}(times/hr) = \frac{(F_{Supp} \times 60)}{V_C}$$

F_{Air}: Air change frequency (minimum unit: 0.1 times/hr)
F_{Supp}: Flow rate of air supply
V_C: Inner volume of the chamber
(2.5 L, actual measurement value)

6.4.4.5 Measurement of environmental condition

Time of measurement: Start of exposure, 1, 2, 3, and 4 hours after start of exposure (actual: on time, acceptable: before and after 5 minutes)
Measurement position: Exposure port of chamber
Measured items: Temperature and relative humidity
Measurement equipment:
Digital thermo-hygrometer (CTH-1100: CUSTOM Corp.)

6.4.5 Dose setting

The target concentration of exposure concentration in this study is set at 2500 ppm to examine whether the fifty percent lethal concentration of the test substance to be below 2500 ppm or not, which is the threshold concentration for deleterious substance in the criteria for poisonous and deleterious substance in Poisonous and Deleterious Substances Control Act in Japan.

[redacted]

6.5 Group constitution

Group name	Number of animals (animal number)	
	Male	Female
2500 ppm	6 (10101–10106)	6 (50101–50106)

6.6 Observation and measurement

The parameters listing in the following sections were observed and measured. The day of exposure was designated as day 1.

6.6.1 Clinical sign

Daily observation was conducted from the day of exposure to the day of necropsy. The observation frequency was shown below.

·Exposure day: Forth a day (pre-exposure, immediately after the end of exposure, and 1 and 2 hours after the end of exposure)

·Other day: Once a day

6.6.2 Body weight

Body weight was measured with an electrical balance (PB-3002S: Mettler Toledo International Inc.) on the following schedule.

·Measurement day: Before exposure on day 1, day 2, day 4, day 8, and day 15

6.6.3 Pathological examination

6.6.3.1 Necropsy

After observation on day 15, all animals were subjected to necropsy after the euthanization by exsanguination from the abdominal aorta after anesthesia by intraperitoneal injection of thiopental sodium (Ravonal: Mitsubishi Tanabe Pharma Corp.).

6.6.3.2 Collection, preservation of organs and tissues, and histopathological examination

Any tissues or organs were not collected, preserved, or subjected to histopathological examination, since there were no gross abnormalities.

6.7 Statistical analysis

None of statistical analysis was conducted.

6.8 Usage of computer system

6.8.1 Computer system

Safety Study System (tsPharma LabSite: Fujitsu Ltd.)

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[redacted]

6.8.2 Protocol number of computer system

[redacted]

The scopes of the data collection and its schedule, etc. were registered in the computer system protocol.

[redacted]

7. Results

7.1 Inhalation exposure

7.1.1 Exposure concentration

Fig. 2 shows the exposure concentration temporal monitoring result, Table 1 shows the measurement results of exposure concentration.

Actual exposure concentration was 2520 ppm to the target concentration of 2500 ppm.

The concentration homogeneity was confirmed, since the coefficient variation among 3 positions was 1.1%; this was within the criterion of the homogeneity, which was to be 15% or below.

The temporal concentration stability was confirmed, since the coefficient variation of the exposure concentration was 1.7% and the relative error was -1.2%–2.0%; these were within the criteria of the stability, which were to be 10% or below on the coefficient variation and within ±10% on the relative error. Moreover, concentration stability was confirmed in the temporal monitoring result.

Nominal concentration was 2570 ppm.

7.1.2 Air flow rate of chamber

Table 2 shows the measurement results of the test atmosphere supply and exhaust of the chamber.

Air flow rate of test atmosphere supply was 16.0 L/min, and exhaust flow rate was 14.0 L/min respectively. It was confirmed that they were intended ranges. Air change frequency of the chamber was 384 times/hr.

7.1.3 Environmental condition

Table 3 shows the results of temperature and relative humidity measurements during exposure.

Temperature was 21.2°C–21.8°C, relative humidity was 18.4%–19.5%. Temperature was within the acceptable range of animal housing. Since pressurized air used for the test atmosphere preparation was dry, relative humidity was low and was out of the acceptable range of animal housing (35.0%–75.0%). However, it was reported that the low relative humidity did not have an influence to the results of inhalation toxicity study [1]. Therefore, it was concluded that the test results were not affected.

7.2 Observation and measurements of animals

7.2.1 Clinical sign

Table 4 shows the results of clinical observations.

There were no abnormalities in any males or females until termination of the observation period on day 15.

[redacted]

7.2.2 Body weight

Table 5 and Appendix 1 show the results of body weight measurements.
Body weight loss was noted on day 2 from day 1. Body weight increased on day 4, and continued to gain throughout the remainder study day in males and females.

7.2.3 Pathological examination

Table 6 shows necropsy findings.
There were no gross abnormalities in any males or females.

[redacted]

8. Discussion and conclusion

This study evaluated the acute inhalation toxicity of [redacted] in rats.

The actual exposure concentration was 2520 ppm.

The test substance exposure resulted in no death or no clinical abnormalities. A decrease in body weight was observed on day 2. On day 4, body weight increased and continued to gain throughout the remainder study day. No gross abnormalities were observed at the necropsy after the observation period.

From the above results, it is concluded that the 50% lethal concentration (LC50) of [redacted] is more than 2520 ppm as the actual exposure concentration.

[redacted]

9. Reference

1. Pauluhn J and Mohr U. Repeated 4-week inhalation exposure of rats: effect of low-, intermediate, and high-humidity chamber atmospheres. Exp Toxicol Pathol. 1999 Feb;51(2):178-87.

[redacted]

10. Study notes

10.1 Deviation from study protocol

None

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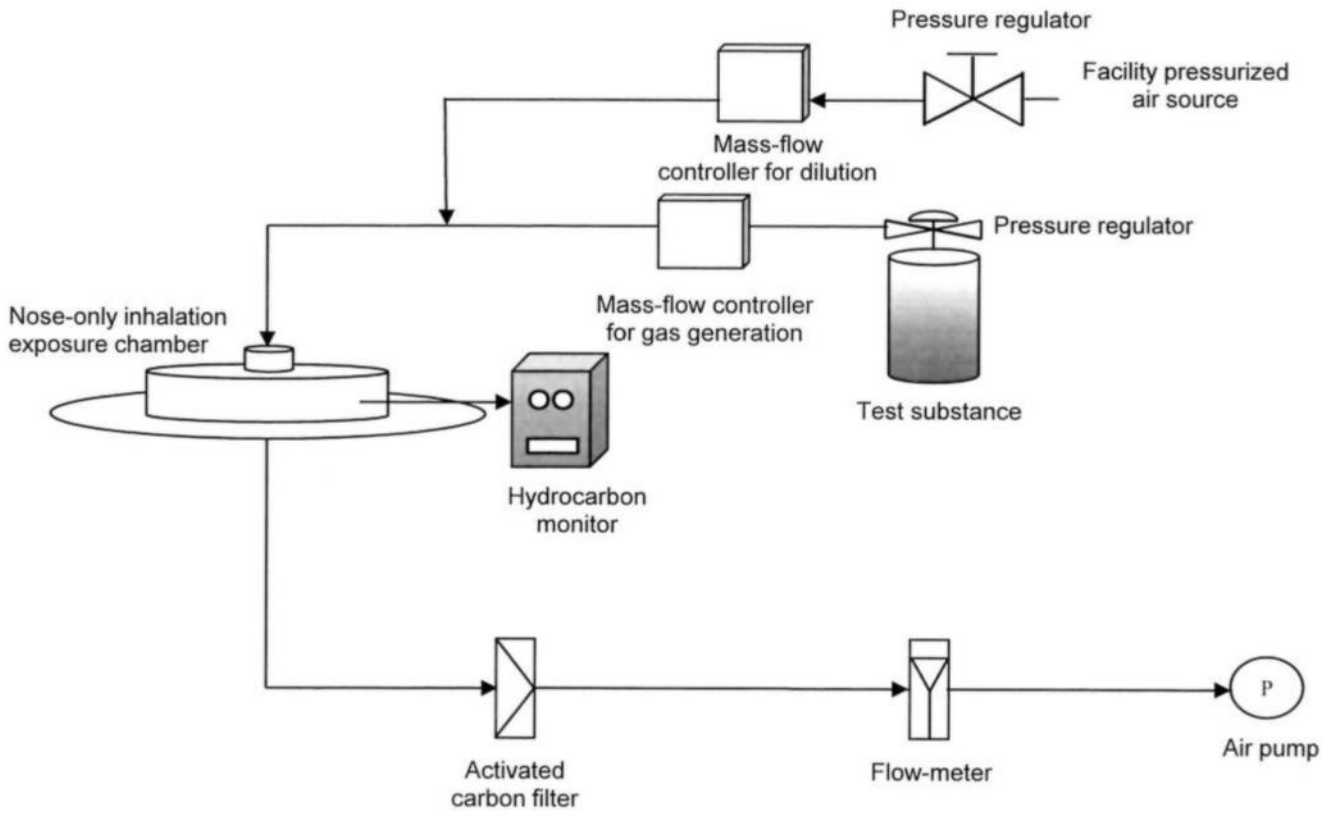

Fig. 1 Inhalation Exposure System

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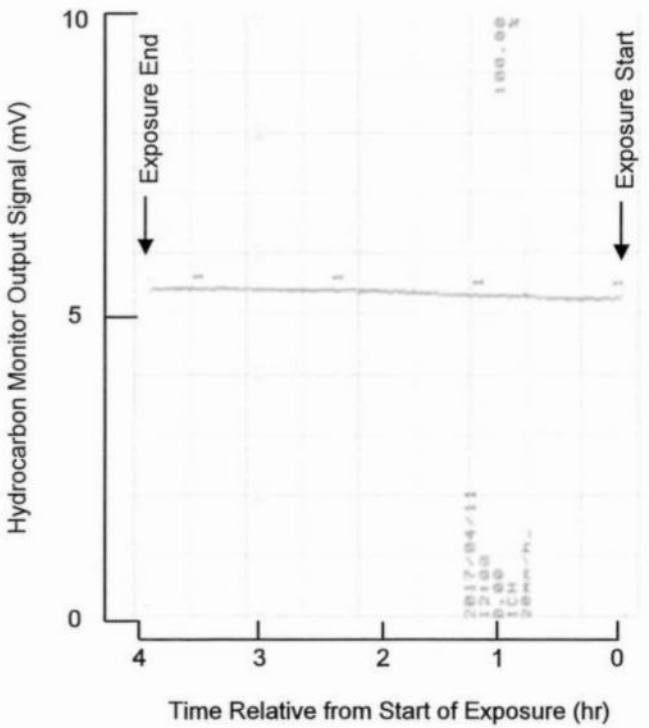

Fig. 2 Hydrocarbon Concentration Monitoring Result - 2500 ppm

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Table 1 Exposure Concentration

Time Relative to Start of Exposure

Target Exposure Concentration	Exposure Concentration (ppm)				Spatial Concentration Homogeneity[1]	Temporal Concentration Stability				Nominal Concentration (ppm)
						R.E.[2]			C.V.[3]	
	0.5 hr	2 hr	3.5 hr	Mean		0.5 hr	2 hr	3.5 hr		
2500 ppm	2570	2510	2490	2520	1.1%	2.0%	-0.4%	-1.2%	1.7%	2570

1: Coefficient variation of concentration between 3 exposure ports (acceptable range: ≤15%)

2: Relative error of exposure concentration to mean concentration (acceptable range: ±10%)

3: Coefficient variation of concentration of test atmosphere (acceptable range: ≤10%)

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Table 2 Inhalation Chamber Air Flow Rate [redacted]

Item	Target Exposure Concentration	Time Relative to Start of Exposure (hr)							Minimum	Maximum
		Pre[1]	0	1	2	3	4	Post[2]		
Test Atmosphere Supply (L/min)	2500 ppm	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0
Test Atmosphere Exhaust (L/min)	2500 ppm	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0
Ventilation Frequency (times/hr)	2500 ppm	384	384	384	384	384	384	384	384	384

1: Pre-exposure; start of test atmosphere generation
2: Post-exposure; end of test atmosphere generation

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Table 3 Environmental Condition of Test Atmosphere

Item	Target Exposure	Time Relative to Start of Exposure (hr)					Minimum	Maximum
		0	1	2	3	4		
Temperature (°C)	2500 ppm	21.6	21.8	21.2	21.2	21.3	21.2	21.8
Relative Humidity (%)	2500 ppm	18.5	19.2	18.4	19.5	19.0	18.4	19.5

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[redacted]

Table 4 Clinical Sign

Sex	Test article	Dose (ppm)	Animal number	Mode of death(day)	Findings	(Frequency or time)	Treatment 1(Before)	1(Just)	1(1h)	1(2h)	2(Day)	3(Day)	4(Day)
Male	[redacted]	2500	10101	SS (15)			-	-	-	-	-	-	-
			10102	SS (15)			-	-	-	-	-	-	-
			10103	SS (15)			-	-	-	-	-	-	-
			10104	SS (15)			-	-	-	-	-	-	-
			10105	SS (15)			-	-	-	-	-	-	-
			10106	SS (15)			-	-	-	-	-	-	-
Female	[redacted]	2500	50101	SS (15)			-	-	-	-	-	-	-
			50102	SS (15)			-	-	-	-	-	-	-
			50103	SS (15)			-	-	-	-	-	-	-
			50104	SS (15)			-	-	-	-	-	-	-
			50105	SS (15)			-	-	-	-	-	-	-
			50106	SS (15)			-	-	-	-	-	-	-

SS : Scheduled necropsy

Sanitized

[redacted]

Table 4 Clinical Sign (Continued)

Sex	Test article	Dose (ppm)	Animal number	Mode of death(day)	Findings	(Frequency or time)	Treatment 5(Day)	6(Day)	7(Day)	8(Day)	9(Day)	10(Day)	11(Day)
Male	[redacted]	2500	10101	SS (15)			-	-	-	-	-	-	-
			10102	SS (15)			-	-	-	-	-	-	-
			10103	SS (15)			-	-	-	-	-	-	-
			10104	SS (15)			-	-	-	-	-	-	-
			10105	SS (15)			-	-	-	-	-	-	-
			10106	SS (15)			-	-	-	-	-	-	-
Female	[redacted]	2500	50101	SS (15)			-	-	-	-	-	-	-
			50102	SS (15)			-	-	-	-	-	-	-
			50103	SS (15)			-	-	-	-	-	-	-
			50104	SS (15)			-	-	-	-	-	-	-
			50105	SS (15)			-	-	-	-	-	-	-
			50106	SS (15)			-	-	-	-	-	-	-

SS : Scheduled necropsy

Sanitized

[redacted]

Table 4 Clinical Sign (Continued)

Sex	Test article	Dose (ppm)	Animal number	Mode of death(day)	Findings	(Frequency or time)	Treatment 12(Day)	13(Day)	14(Day)	15(Day)
Male	[redacted]	2500	10101	SS (15)			-	-	-	-
			10102	SS (15)			-	-	-	-
			10103	SS (15)			-	-	-	-
			10104	SS (15)			-	-	-	-
			10105	SS (15)			-	-	-	-
			10106	SS (15)			-	-	-	-
Female	[redacted]	2500	50101	SS (15)			-	-	-	-
			50102	SS (15)			-	-	-	-
			50103	SS (15)			-	-	-	-
			50104	SS (15)			-	-	-	-
			50105	SS (15)			-	-	-	-
			50106	SS (15)			-	-	-	-

SS : Scheduled necropsy

Sanitized

[redacted]

Table 5 Body Weight (g) - Summary (mean ± S.D.)

Sex	Test article	Dose (ppm)	No. of animals	Initial B.W.	Day 2	4	8
Male	[redacted]	2500	6	315.55 ± 7.03	305.39 ± 8.02	325.11 ± 9.83	349.46 ± 8.94
Female	[redacted]	2500	6	218.49 ± 8.58	210.94 ± 8.52	225.12 ± 11.45	233.98 ± 12.52

Sex	Test article	Dose (ppm)	No. of animals	Day 15
Male	[redacted]	2500	6	394.01 ± 11.89
Female	[redacted]	2500	6	251.12 ± 16.32

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Table 6 Necropsy Findings

Sex	Male						Female					
Group	2500 ppm						2500 ppm					
Animal Number	1	1	1	1	1	1	5	5	5	5	5	5
	0	0	0	0	0	0	0	0	0	0	0	0
	1	1	1	1	1	1	1	1	1	1	1	1
	0	0	0	0	0	0	0	0	0	0	0	0
	1	2	3	4	5	6	1	2	3	4	5	6
Reason of Necropsy	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS
	D15	D15	D15	D15	D15	D15	D15	D15	D15	D15	D15	D15
No Abnormalities	P	P	P	P	P	P	P	P	P	P	P	P

Reason of necropsy: SS; scheduled sacrifice
D: study date
P: finding present

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[redacted]

Appendix 1 Body Weight (g)

Sex	Test article	Dose (ppm)	Animal number	Mode of death(day)	Initial B.W.	Day 2	4	8	15
Male	[redacted]	2500	10101		317.0	310.6	323.8	347.3	387.5
			10102		314.8	306.1	327.7	346.6	387.3
			10103		322.4	313.9	337.3	363.9	416.8
			10104		305.2	294.0	310.3	340.6	389.4
			10105		310.4	297.3	318.5	342.1	385.9
			10106		323.6	310.5	333.0	356.3	397.2
Female	[redacted]	2500	50101		216.2	209.9	216.6	233.4	253.9
			50102		211.8	207.8	225.1	230.2	252.8
			50103		220.8	208.7	228.9	232.8	243.4
			50104		215.7	214.8	226.8	235.0	264.3
			50105		211.8	199.3	209.9	216.7	223.3
			50106		234.6	225.0	243.4	255.7	268.9

Sanitized

[redacted]

Quality Assurance Statement

Title: An Acute Inhalation Toxicity Study of [redacted] in Rats
Study No.: [redacted]

This study was carried out in accordance with the following standard. I hereby that this final report faithfully describes the method and results in this study. The inspection and reporting are as follows.

OECD Principles of Good Laboratory Practice (as revised in 1997)

Inspections	Inspection date	Reporting Date	
		to the Study Director	to the Management
Study Protocol			
Study protocol	Mar. 31, 2017	Mar. 31, 2017	Mar. 31, 2017
Computer protocol	Apr. 10, 2017	Apr. 10, 2017	Apr. 10, 2017
Amendment No. 1	Apr. 14, 2017	Apr. 14, 2017	Apr. 14, 2017
Study Procedure			
Receipt of animals, body weight measurements	Apr. 05, 2017	Apr. 05, 2017	Apr. 05, 2017
Test atmosphere generation, inhalation exposure, clinical observations	Apr. 11, 2017	Apr. 11, 2017	Apr. 11, 2017
Exposure concentration measurements	Apr. 11, 2017	Apr. 11, 2017	Apr. 11, 2017
Environmental condition measurements	Apr. 11, 2017	Apr. 11, 2017	Apr. 11, 2017
Necropsy	Apr. 25, 2017	Apr. 25, 2017	Apr. 25, 2017
Raw data, Final report			
Raw data, draft report	May 18–19, 2017	May 19, 2017	
(re-inspection)	May 19, 2017	May 19, 2017	May 19, 2017
Raw data, final report	Jun. 05, 2017	Jun. 05, 2017	Jun. 05, 2017

Quality Assurance Manager: Signed Date: June 5, 2017

Terumi Ishimaru
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